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ABPA diagnosis in cystic fibrosis patients: the clinical utility of IgE specific to recombinant *Aspergillus fumigatus* allergens

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Abstract

Objective: Allergic bronchopulmonary aspergillosis (ABPA) is a complicating factor of cystic fibrosis which can result in a devastating combination as lung disease progresses. The overlap between the signs and symptoms of the two conditions make diagnosis problematic, even if standardized criteria are used. The objective of this study was to identify, in a group of cystic fibrosis patients, cases of ABPA by assaying IgE specific to recombinant *Aspergillus fumigatus* antigens and to compare the method with the Cystic Fibrosis Foundation diagnostic criteria.

Methods: Fifty-four patients, aged 2 to 20, presenting characteristics that could occur with ABPA in isolation, were systematically assessed based on the following: clinical data, a chest CT scan, immediate hypersensitivity skin test for *A. fumigatus*; total serum IgE assay, RAST for *A. fumigatus* and serum IgE specific for the recombinant allergens Asp f1, f2, f3, f4 and f6.

Results: Thirty-nine patients were eligible for the study. Thirty-two of these were investigated. Sensitization to *A. fumigatus* was observed in 34%. Both the Cystic Fibrosis Foundation criteria and the recombinant antigen specific IgE assay, defined three patients as suffering from ABPA; however, only two of these patients were diagnosed by both methods.

Conclusions: The detection of *A. fumigatus* recombinant antigen specific IgE was a useful tool for the early detection of sensitization and diagnosis of ABPA. Nevertheless, diagnostic confirmation cannot be divorced from clinical findings and before this method can be used for ABPA diagnosis, for detecting relapses and for defining cure criteria longitudinal studies with larger numbers of patients are required.

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Introduction

Allergic bronchopulmonary aspergillosis (ABPA) is a lung hypersensitivity disease mediated by an allergic late-phase inflammatory response to certain antigens of *Aspergillus fumigatus*.[1] In patients with cystic fibrosis (CF), the presence of atopic disease, bronchial hypersecretion and a certain difficulty in removing microorganisms from the respiratory tree can be listed as factors that facilitate colonization and possible sensitization to *A. fumigatus*.[2]

There is bronchial colonization by *A. fumigatus* in 12 to 57% of CF patients,[3] and sensitization to the fungus may vary from 30 to 51% in all cases.[4] The prevalence of ABPA varies greatly, from 1 to 15%,[5,6] and increases in line with patient age.[7] The duration of endobronchial colonization by *Pseudomonas aeruginosa* and colonization by *Stenotrophomonas maltophilia* represent risk factors for sensitization to *Aspergillus* and ABPA, respectively.[8] Allergic bronchopulmonary aspergillosis is a complicating factor of cystic fibrosis which can result in a devastating combination as lung disease progresses,[9,10] and early diagnosis is of fundamental importance to preventing pulmonary damage and functional deterioration.[11]

Despite recent advances in the understanding of ABPA immunopathology, diagnosis in patients with CF is still imprecise.[12] Even with the adaptation of the classic ABPA diagnostic criteria [13] for use with CF patients,[4,14] doubt

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very often remains when differentiating patients with ABPA from those who are only sensitized to the fungus.

The development of recombinant components of *A. fumigatus* antigens has made it possible to identify IgE specific to some of these allergens. It has been demonstrated that patients with ABPA react to a larger number of the recombinant allergens in this panel, some of which – r Asp f2, f4 and f6 – are considered specific to ABPA patients.[15-18] This technique therefore appeared to be very promising for ABPA diagnosis.

The objective of this study was to identify cases of ABPA in a sample of CF patients, employing the technique of detecting IgE specific to the recombinant *A. fumigatus* allergens r Asp f2, f4 and f6 and to compare the results from this method with diagnoses based on the Cystic Fibrosis Foundation (CFF) standardized criteria.[14]

Methods

This was a cross-sectional study carried out during the routine clinical assessments of patients with diagnoses of CF, made in accordance with the CFF consensus statement criteria.[19] These patients were being treated at the Pediatric Pulmonology Section, Child Institute, at Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo (HC-FMUSP) between June 26 and October 10, 2001.

Inclusion criteria

Patients were recruited if they exhibited any characteristic that, in isolation, could indicate ABPA. Therefore, children and adolescents aged 2 to 20, were included in the presence of any of the following characteristics:

- Personal history of atopic disease with typical manifestations of any of the following diseases: rhinitis, conjunctivitis, dermatitis, angioedema, insect bite allergy, urticaria, food allergy and asthma;
- History of wheezing, even in the absence of asthma;
- The presence of one or more sputum and/or oropharynx cultures positive for *Aspergillus* during the 2 years prior to enrollment on the study protocol;
- Serum IgE assay result over 500 UI/ml;
- Immediate hypersensitivity skin test positive for *A. fumigatus*;
- Clinical and functional deterioration during the previous 6 months without responding to treatment with antimicrobials.

The clinical and laboratory assessment consisted of: characterization of the type of endobronchial colonization based on the results of previous cultures, Shwachman score,[20] and spirometry, which was measured only for patients older than 6 years, in accordance with the standards laid down by the American Thoracic Society (ATS),[21] using a Warren Collins® bell spirometer or a Multispiro® portable spirometer. The Shwachman score is an objective method for evaluating the clinical severity of patients with CF and includes data on physical limitations, physical examination findings, nutritional status and the extent of radiological findings. At our clinic the score is calculated annually for all patients. The tests included in the research protocol are part of the annual laboratory assessments. Annual chest radiography has been substituted by tomography with a volumetric protocol of 10 mm slices at 10 mm increments, which results in an effective radiation dose that is much lower than the conventional examination. Immediate hypersensitivity skin testing was performed using the puncture technique, as previously described elsewhere, using standardized extract (International Pharmaceutical Immunology S.A. – IPI, Spain®), 10 mg/ml histamine positive control and a negative control of phenolated solution at 0.5% and glycerinated solution.[22] Tests were considered positive if the wheal was 3 mm or larger, in the presence of a negative control of 0 and a positive control greater than or equal to 3 mm.

Testing (RAST) for serum IgE specific to *A. fumigatus* and for serum IgE specific to the *A. fumigatus* recombinant allergens (ImmunoCAPs) – r Asp f1 (IgERm218), f2 (IgERm219), f3 (IgERm220), f4 (IgERm221) and f6 (IgERm222) – was carried out at the Laboratório Fleury de São Paulo using the automated UniCAP® enzyme immunofluorescence technique in a UniCAP 100 (Pharmacia-Diagnostics) machine. ImmunoCAPs were produced and employed for the titration of allergen specific serum IgE in accordance with specifications described by Crameri et al.[23] The results were converted into standardized RAST classes from 0 to 4, in accordance with the manufacturer's instructions (Table 1). For data analysis, clinical diagnosis of ABPA was made according to the CFF minimum criteria:[14] acute or subacute clinical deterioration not attributable to another etiology, total serum IgE concentration of >500 IU/mL (1200 ng/mL), immediate cutaneous reactivity to *Aspergillus* test wheal of >3 mm or demonstration of IgE antibody to *A. fumigatus*, plus at least one of: precipitins to *A. fumigatus* or in vitro demonstration of IgG antibody to *A. fumigatus,* new or recent abnormalities on chest radiography or chest CT with bronchiectasis that did not improve with antibiotics and standard physiotherapy. Precipitin assay is not available at our service and for this reason was not employed.

A diagnosis of ABPA by recombinant antigen specific IgE was defined as assay results greater than or equal to the lower cutoff point for class 3 for IgE antibodies to the *A. fumigatus* antigens f2, f4 and f6.[24] Sensitization to *A. fumigatus* was defined as the presence of a positive

immediate hypersensitivity skin test and/or RAST for *A. fumigatus* and/or serum IgE specific to Asp f1 above 0.35 kU_A/l.

The statistical analysis used Fisher's exact test, the chi-square test or the Mann-Whitney tests to compare groups. Sensitivity and specificity calculations were made using 2 x 2 contingency tables.

This project was approved by the medical Research Ethics Committee at the Hospital das Clínicas, Universidade de São Paulo. Patients and their parents or tutors were informed and gave their consent.

Table 1 - Classification of results of allergen specific IgE assay

Specific IgE class	kU_A/l	Specific IgE level
0	Below 0.35	Absent or undetectable
1	0.35-0.69	Low
2	0.7-3.49	Moderate
3	3.5-17.49	High
4	17.5-49	Very high
5	50-99	Very high
6	100 or more	Very high

Results

Fifty-four CF patients were assessed during the study period and, of these, 39 were considered eligible, although just 32 agreed to participate and to attend for sample collection and testing. The mean age of the study group was 9.8 years (±3.8) and mean Shwachman score was 65 (±15) (Table 2). Eleven patients were identified as sensitized to *A. fumigatus* (34%). Just three of them had recombinant antigen specific IgE profiles that were compatible with ABPA. Table 3 contains the laboratory data for patients with detectable IgE specific for any of the recombinant antigens or with a diagnosis of ABPA made according to the CFF consensus criteria.

Table 2 - Clinical and laboratory data for the study group

Parameter	Mean ± DP	95%CI	Median
Age	9.8±3.8	8.4-11.2	10.1
Score	65±15	59.6-70.4	65
Absolute number of eosinophils	307±250	215-398	240
Serum IgE	510±908	151-869	154

DP = standard deviation; 95%CI = 95% confidence interval for mean.

There was no relationship between the severity of underlying disease, expressed by the Shwachman score, degree of bronchial obstruction or chronic colonization by *Pseudomonas aeruginosa* with ABPA diagnosed by recombinant antigen specific IgE. A combination of IgE > 500, positive RAST and skin test positive for *A. fumigatus* had a sensitivity of 75%, specificity of 94%, positive predictive value of 50% and negative predictive value of 94% for identifying those ABPA cases diagnosed by recombinant antigen specific IgE.

Three patients were identified as having ABPA according to the CFF criteria.[14] Two of them had IgE specific to the f2 and f4 fractions and therefore compatible with ABPA. The other patient exhibited only sensitization with IgE specific to the f1 fraction (Table 3).

All patients had CT abnormalities suggestive of bronchiectasis. This examination does not, therefore, differentiate patients by presence or absence of ABPA.

Discussion

In this population of 32 children and adolescents with CF, two patients with ABPA and 11 individuals sensitized to the fungus were identified by means of skin tests, *A. fumigatus* specific IgE assay (RAST) and by testing for IgE specific to the recombinant allergens Asp f1, f2, f3, f4 and f6.

It was the difficulty of diagnosing ABPA in CF patients that motivated this study, which was the first opportunity to research IgE specific to *A. fumigatus* recombinant antigens in our country. The frequency of sensitized individuals was 34%. When the results of the recombinant antigen specific IgE assay were compared with diagnoses made according to the CFF consensus criteria with the benefit of knowledge of the individual clinical histories of each patient, two patients could be identified with a definitive diagnosis of ABPA. There were, therefore, 6.4% of the sample who had a diagnosis of ABPA.

The prevalence of sensitization to the fungus and of ABPA itself varies across CF treatment centers,[4,5] and there are no national data on these prevalence rates. Estimates and descriptions of the prevalence of ABPA and of sensitization to *A. fumigatus* based only on classic criteria, even in developed countries, may be underestimated, since the difficulties in diagnosing ABPA in CF are universal.[10] Cunningham et al.[12] surveyed the criteria used to diagnose ABPA in specialist clinics in the United Kingdom. Tests such as precipitins, specific IgE and skin tests for *A. fumigatus*, eosinophil count in peripheral blood, total IgE and chest radiography were performed routinely and, on average, the centers performed four of the six tests annually. Nevertheless, there was a large variation in the criteria used to confirm diagnosis in order to start treatment.

Table 3 - Laboratory data for patients with detectable recombinant antigen specific IgE and/or diagnosis of ABPA by the CFF consensus criteria

Patient	CFF *	RAST *Aspergillus fumigatus*	IgE r Asp f1	IgE r Asp f2	IgE r Asp f3	IgE r Asp f4	IgE r Asp f6
V.B.A.	no	12 (3)	1.1 (2)	**3.5 (3)**	< 0.35 (0)	2.4 (2)	0.6 (1)
R.S.F.	ABPA	29 (4)	1.8 (2)	**14.4 (3)**	26.4 (4)	1.4 (2)	< 0.35 (0)
C.P.O.	ABPA	39.1 (4)	6.8 (3)	**41.2 (4)**	3.3 (2)	**5 (3)**	< 0.35 (0)
A.P.P.R.	ABPA	72.6 (5)	0.5 (1)	< 0.35 (0)	< 0.35 (0)	< 0.35 (0)	< 0.35 (0)
J.K.P.	no	7.6 (3)	3.6 (3)	2.9 (2)	< 0.35 (0)	< 0.35 (0)	0.4 (1)
T.A.A.C.	no	< 0.35 (0)	6.2 (3)	< 0.35 (0)	< 0.35 (0)	0.9 (1)	< 0.35 (0)

ABPA = allergic bronchopulmonary aspergillosis; CFF = Cystic Fibrosis Foundation; RAST = IgE specific to *A. fumigatus*.
* Diagnosis of ABPA by the CFF.[14] Precipitin assay was not used.
IgE assay expressed in kU_A/l (class). Laboratory results in **bold** are compatible with ABPA.
Patients R.S.F. and C.P.O. were defined as having ABPA and were treated. Patient V.B.A. was defined as a carrier of previous ABPA ("serological scar").

Prevalence data therefore depends on which criteria and laboratory tests are considered when defining sensitization and ABPA.[14] It is also known that immunological test results can fluctuate over time, with intra-individual variation.[25] Additionally, there are reports of patients who had presented sensitization and then ceased to have test results compatible with sensitization during follow-up.[26]

In this sample, the association of positive skin test for *A. fumigatus* hypersensitivity, absolute number of eosinophils in peripheral blood over 500/mm^3, serum IgE assay result over 500 UI/ml and positive RAST for *A. fumigatus*, offered good specificity for ABPA diagnosis. In addition to the importance of these tests for periodic screening of patients at risk from ABPA, the combination is useful because these tests are simpler and more likely to be available than the test for *A. fumigatus* recombinant antigen specific IgE.

In patients with CF, the relationship between high serum IgE and ABPA has already been reported by several authors, and this is one of the laboratory variables included in the classic diagnostic criteria.[5,27] What is peculiar to CF patients is that these IgE levels may not be so high,[28,29] as the CFF consensus emphasizes in suggesting that annual ABPA screening be performed if there is a strong clinical suspicion even when IgE < 500 UI/ml.[14] The level of serum IgE varies according to the phase of disease activity,[1] and reduced levels after treatment with corticosteroids is another factor to be taken into account when diagnosing ABPA.[4,5]

Banerjee et al.[17] demonstrated that CF patients with ABPA exhibit significantly higher levels of IgE specific to the Asp f2 antigen, when compared with controls, with 100% specificity. This antigen appears to be involved with the adherence of the fungus to the extracellular matrix. The native and recombinant forms of this antigen were assessed and considered immunologically comparable and useful for specific diagnosis of ABPA. In ABPA fungus grows in the airways and the immune reaction that follows results in the destruction of these microorganisms, with exposure of intracellular antigens. It is believed that detecting IgE specific to these intracellular antigens of *Aspergillus* indicates the disease. In contrast, immunoresponse against proteins secreted after germination of spores, such as r Asp f1 and r Asp f3, is observed both in individuals with ABPA and in those who are sensitized. Preliminary studies of the cellular distribution of the r Asp f4 antigen indicate that it is a secreted protein.[30] The r Asp f6 antigen has been identified as a protein specific to the hyphae of *Aspergillus*, and these hyphae are only present in ABPA.[31] Therefore, identification of IgE specific to the r Asp f4 and r Asp f6 antigens can also be used as a marker specific to ABPA.[30] In our sample, both ABPA patients' assay results for IgE specific to r Asp f6 were defined as class I, in common with what has been reported by other researchers who observed lower r Asp f6 specific IgE levels, when compared with the other antigens.[15,30,32]

When the ABPA diagnosis made by assaying IgE specific to recombinant antigens was compared with the

diagnosis made by the CFF criteria, there was only agreement on two patients, and therapeutic intervention was indicated for them. After analyzing the clinical progress of the two patients who had been diagnosed by one method, but not by the other, we concluded that:

- Patient V.B.A., with a profile of IgE for recombinant antigens suggestive of ABPA and a previous history of treated ABPA, was considered a probable case of a serological scar, which was also described in an epidemiological study by Geller.[4] When the profiles of IgE to recombinant antigens are assessed individually, at different points of the clinical course, IgE specific to r Asp f3 appears to remain high during remission;[24,33] although this behavior was not observed in our patient.
- Patient A.P.P.R. fulfilled the CFF consensus criteria for a diagnosis of ABPA, but did not have a profile of IgE for recombinant antigens compatible with the disease. Laboratory data only suggested sensitization to the fungus. This is an understandable situation because of the overlapping of the clinical signs of the two diseases, in a severe case of CF, with advanced lung disease and total serum IgE = 4,353 UI/ml.

Cutoff values have not yet been defined for these assays of IgE for recombinant antigens, but published studies all adopt values compatible with a minimum of class III (over 3.5 kU_A/l).[24] Knutsen et al.,[33] studied 15 patients with ABPA – 23 sensitized and 19 CF controls – demonstrating that IgE specific to the f4 fraction has the greatest sensitivity and specificity for detecting ABPA. Patient T.A.A.C. presented detectable levels of IgE to r Asp f4, but at a low level (class 1), and patient J.K.P. had classes I and II IgE specific for r Asp f6 and f2, respectively, both without clinical status of ABPA. These patients may represent an initial form of ABPA, with minimal abnormalities mainly in laboratory test results. These patients are undergoing clinical surveillance and annual laboratory screening due to the possibility of ABPA in the future.

Casaulta et al.[24] performed a longitudinal analysis, over approximately 3 years, of the clinical and laboratory data of 23 patients with ABPA, comparing them with *Aspergillus* sensitized and unsensitized controls. These patients presented high levels of IgE specific to r Asp f4 and r Asp f6, and an association with total serum IgE over 1,000 UI/ml allowed ABPA to be confirmed with high specificity (100%) and sensitivity (64%). Under this treatment, specific and total IgE levels were reduced but did not normalize, and pulmonary function remained stable despite the treatment. Testing for IgE to recombinant antigens of *A. fumigatus* was considered useful for identifying patients at increased risk of ABPA, but laboratory data was not sufficient to monitor treatment.

Allergic bronchopulmonary aspergillosis is an infrequent complication of CF and diagnostic confirmation remains a challenge. Despite the efforts directed at establishing diagnostic criteria adapted to CF patients, diagnostic failures still occur. We performed a systematic investigation comparing two distinct strategies, the CFF criteria and the detection of IgE specific to recombinant antigens, and found that the diagnostic conclusions were discordant. Assaying IgE to recombinant antigens of *A. fumigatus* appears to us to be a promising technique for the early detection of sensitization to the fungus and of ABPA itself, perhaps during a phase with little clinical pulmonary symptomology. However, the true utility of this laboratory method to the clinical practice has not yet been defined. Despite the high specificity previously described for this laboratory method, neither diagnostic confirmation nor the treatment can be divorced from the clinical condition of the patients. Further longitudinal studies with larger numbers of patients remain necessary to achieve diagnosis during the diverse stages of the disease and for detecting relapses and establishing cure criteria after therapeutic intervention.

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